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(54) Title: CALCIUM, TRACE MINERAL, VITAMIN D AND DRUG THERAPY COMBINATIONS

(57) Abstract

Nutritional mineral supplements comprising calcium citrate malate, salts of manganese, copper and zinc and a member or members selected from the group of vitamin D or its metabolites or precursors and drug therapies consisting of calcitonin, editronate, diphosphonates and amino-diphosphonates are disclosed. Estrogen can also be used with these supplements. These supplements, which provide at least 25 % RDA of the calcium, trace minerals and vitamins, are used in addition to the normal diet. These supplements are useful for increasing bone growth and for treating age-related bone loss in humans and animals. The supplements may be in the form of solid tablets, liquid unit dosage forms, or in a beverage.

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CALCIUM, TRACE MINERAL, VITAMIN D AND DRUG THERAPY COMBINATIONS

TECHNICAL FIELD

The present invention relates to nutritional and therapeutic improvements in calcium supplements containing trace minerals, in particular copper, manganese and zinc and vitamin D, estrogen, calcitonin or Didronel or diphosphonate drug therapies. These supplements are useful for increasing bone growth and treating age-related bone loss. They can be used in conjunction with foods and beverages or taken as an oral solid or liquid supplement. The invention also relates to a method of building bone or treating bone loss in osteoporosis patients, post-menopausal women and/or elderly men.

BACKGROUND OF THE INVENTION

Vitamin and mineral supplements for human and veterinary use are commonplace. Some diets, heavy physical exercise and disease conditions may require the intake of considerable quantities of minerals and vitamins apart from those generally obtained through what otherwise would be considered a normal diet. Vitamin and mineral supplementation is important primarily for those who have inadequate diets, including growing children. Older adults have an additional need for calcium to help prevent the bone loss which occurs as a normal consequence of the aging process. In particular, postmenopausal women need additional calcium due to hormonal changes which can accelerate the bone loss rate leading to a further diminishment in bone mass.

The trace minerals which affect bone growth are copper, zinc and manganese. Supplementation of the diet with these minerals along with a highly bioavailable source of calcium is highly desirable. Commercially available mineral supplements are useful in many circumstances where increased mineral intake is desirable. Most of these

multi-vitamin and multi-mineral tablets are low in calcium, requiring separate supplementation with calcium sources. In addition, not all calcium sources are equal in terms of bioavailability and absorption. The addition of the vitamin D, calcitonin, estrogen and/or other therapy (editronate or diphosphonates) makes the supplementation more complex since three pills or more could be involved. It would be more convenient if all of the minerals and drugs and/or vitamins could be administered conjointly in a convenient and/or pleasant tasting form which would not require extra attention, planning and implementation by the user. This could be done in the form of foods and beverages as well as in the form of tablets.

There are well-recognized problems associated with adding both minerals and vitamins to foods and beverages. Some of these are taste; calcium tends to be chalky in flavor. In addition, the solubility of many calcium sources prevents them from being added to many beverages. Others are interactions of the minerals or vitamins with the food or beverage which affects the stability and/or the bioavailabilty of the product. This invention provides a means for making such product.

This invention also relates to methods of building bone in humans and other animals, i.e., for the treatment of age-related bone loss and related disorders. In particular, this invention relates to such methods of treatment by administration of certain calcium salts and the minerals, copper, zinc and manganese along with vitamin D and/or drug therapy.

Calcium is the fifth most abundant element in the human body. It plays an important role in many physiological processes, including nerve and muscle functions. Not surprisingly, nutritional and metabolic deficiencies of calcium can have broad-ranging adverse effects. Since about 98% to 99% of the body's calcium is found in bone tissues, many of these adverse effects are manifested

through deficiencies in the structure, function and integrity of the skeletal system.

The most common metabolic bone disorder is osteoporosis. Osteoporosis can be generally defined as the reduction in the quantity of bone, either from the reduction in bone formation or the acceleration of bone resorption, in either event the result is a decrease in the amount of skeletal tissue. In general, there are two types of osteoporosis: primary and secondary. "Secondary osteoporosis" is the result of an identifiable disease process or agent. However, approximately 90% of all osteoporosis cases are idiopathic "primary osteoporosis". Such primary osteoporosis includes postmenopausal osteoporosis, age-associated osteoporosis (affecting a majority of individuals over the age of 70 to 80), and idiopathic osteoporosis affecting middle-aged and younger men and women.

For some osteoporotic individuals the loss of bone tissue is sufficiently great so as to cause mechanical failure of the bone structure. Bone fractures often occur, for example, in the wrist, hip and spine of women suffering from postmenopausal osteoporosis. Kyphosis (abnormally increased curvature of the thoracic spine) may also result.

The mechanism of bone loss in osteoporotics is believed to involve an imbalance in the process of "bone remodeling". Bone remodeling occurs throughout life, renewing the skeleton and maintaining the strength of bone. Two reactions are involved, bone loss or resorption and bone growth or accretion. This remodeling occurs in a series of discrete pockets of activity in the bone, called "osteoclasts" and "osteoblasts". Osteoclasts (bone dissolving or resorbing cells) are responsible for the resorption of a portion of bone within the bone matrix, during the resorption process. After resorption, the osteoclasts are followed by the appearance of osteoblasts

(bone forming cells), which then refill the resorbed portion with new bone.

In young healthy adults, the rate at which the osteoclasts and osteoblasts are formed maintains a balance of bone resorption and bone formation. However, as normal consequency of aging an imbalance in this remodeling process develops, resulting in loss of bone at a rate faster than the accretion of bone. As imbalance continues over time the reduction in bone mass and thus bone strength leads to fractures.

Many compositions and methods are described in the medical literature for the "treatment" of osteoporosis. See, for example, R. C. Haynes, Jr. et al., "Agents affecting Calcification", The Pharmacological Basis of Therapeutics, 7th Edition (A. G. Gilman, L. S. Goodman et al., Editors, 1985); and G. D. Whedon et al., "An Analysis of Current Concepts and Research Interest in Osteoporosis", Current Advances in Skeletogenesis (A. Ornoy et al., Editors, 1985). Estrogen is often used to affect the metabolism of calcium by influencing the osteoblast cells. Treatments using fluoride have also been described. However, the utility of such agents may be limited, because of possible adverse side effects. See W. A. Peck, et al., Physician's Resource Manual on Osteoporosis (1987), published by the National Osteoporosis Foundation.

Nutritional therapies for osteoporosis have also been proposed. Many calcium-containing compounds and compositions have been described for use as nutritional supplements. Many commercial preparations are also available, typically containing calcium carbonate or calcium phosphate. Other calcium salts have also been described for use in calcium supplements, including calcium lactate, calcium citrate and calcium gluconate.

US 3,949,098 issued Bangert (assigned Nabisco, 1976) describes a nutritious orange drink concentrate that contains whey protein. The patent suggests the addition of

minor amounts of vitamins and other nutrients which include various cupric salts, manganese salts, zinc salts, as well as calcium salts.

German OLS 2,845,570 issued to E.R.E. (Europe Representation Establishment, 1980) describes a honey containing composition. Honey contains low levels of calcium, manganese, copper as well as trace amounts of magnesium, iron, phosphorous, silicon and nickel. The value of honey as a medicant is undisputed according to this patent application. This application claims a honey containing composition with levarotatory ascorbic acid and citric acid. This patent has issued as US 4,243,794 (1981).

US 4,497,800 issued to Larsen et al (assigned Mead Johnson & Company, 1985) describes a nutritionally complete ready-to-use liquid diet for providing total patient nourishment. The diet contains free amino acids and small peptides, a carbohydrate source, and nutritionally significant amounts of all essential vitamins and minerals, and stabilizers. The minerals include calcium, copper, zinc and manganese, among others. Most of these minerals are given as the gluconate salt.

"Effects of calcium carbonate and hydroxyapatite on zinc and iron retention in postmenopausal women", Dawson-Hughes, Seligson and Hughes, American Journal of Clinical Nutrition, 44, 83-88 (1986) describes the effect of calcium carbonate on whole-body retention of zinc and iron in thirteen healthy post menopausal women. The test meal, including both dry food and a formulated beverage, included calcium, copper and zinc at a level of one-third the usual daily requirement. These are levels normally found in human diets.

US 3,992,555 issued to Kovacs (assigned Vitamins, Inc., 1976) describes food supplements prepared by mixing assimilable iron compounds, vitamins and minerals with a heated edible fat carrier. The minerals include calcium, zinc, copper, and manganese among others.

US 3,950,547 issued to Lamar et al (assigned Syntex Inc, 1976) describes a dietary composition containing peptides and/or amino acids, lipids and carbohydrates in an aqueous emulsion. Suitable minerals for adding at low levels include among others calcium, copper and zinc.

US 4,107,346 issued to Kravitz (1978) describes a dietary salt composition for use as a replacement for salt in foods. The role of copper is described as a component of several enzymes essential for nutrition. The patent further discloses that "spontaneous fractures are common in animals feeding off copper deficient soils or who are given artificially depleted copper diets". (column 4, lines 20-30) Zinc is described as helping in growth, wound healing and improving taste and smell. Clinical symptoms of manganese deficiency have not been observed in man. However, there is no question that manganese is essential for human nutrition. Main manifestations of its deficiency are impaired growth and skeletal abnormalities. Calcium is described as being necessary for blood coagulation, for calcium retention, and for relieving the symptoms of osteoporosis. Examples of salts that contain these four trace minerals are disclosed.

US 4,070,488 issued to Davis (unassigned, 1978) discloses a highly stabilized balanced nutritive composition useful in supplementing the diet of humans and/or animals. This composition contains gelatin. The patent discloses that the sulfhydryl groups of the gelatin can render copper inactive toward ascorbic acid.

US 4,214,996 issued to Buddemeyer et al (R.G.B. Laboratories, 1980) discloses mineral compositions which are very soluble. These compositions contain calcium, phosphorus, zinc, as well as manganese. Not all of the compositions that are described contain all four elements.

US 4,351,735 to Buddemeyer et al (R.G.B. Laboratories, 1982) is related to the '996 patent.

"Nutrients and Nutrition of Citrus Fruits," Citrus Nutrition and Quality, Ting, (American Chemical Society, 1980) discloses the presence of certain trace minerals in orange juice. These include copper, zinc, iron and manganese. Calcium and magnesium are the two major divalent cations in orange juice. The levels of all the minerals are low.

Hungerford et al, "Interaction of pH and ascorbate in intestinal iron absorption," (1983) describes the iron absorption from various food materials. The diet which was low in iron also contained calcium carbonate, manganese sulfate and copper sulfate among others.

US 4,419,369 issued to Nichols et al (assigned Baylor College of Medicine, 1983) describes an improved dietary protein mineral module for infants. An approximate analysis of the material shows the presence of iron, zinc, copper and calcium.

The utility of these known supplements varies. Unlike agents (such as estrogen) which affect the metabolism of bone, nutritional supplements such as calcium, have been thought to merely provide a source of the nutrient (which may or may not be properly absorbed and metabolized). See, for example, B. Riis et al., "Does Calcium Supplementation Prevent Postmenopausal Bone Loss?," New England J. of Medicine, 316, 173-177 (1987); L. Nilas et al., "Calcium Supplementation and Postmenopausal Bone Loss," British Medical Journal, 289, 1103-1106 (1984); and H. Spencer et al., "NIH Concensus Conference: Osteoporosis," Journal of Nutrition, 116, 316 -319 (1986).

It has now been discovered, however, that the administration of mixtures of certain calcium salts and trace minerals are effective for delaying age-related loss of bone (see co-pending application of Smith et al., Serial Number 07/562,773).* Furthermore, it has been separately found that the administration of mixtures of certain calcium salts with vitamin D is also effective for attenuating

(* see publication number on page 32)

age-associated bone loss (see co-pending application of Andon et al, Serial Number (P&G Case 4394). It would be desirable, therefore to have mixed calcium, trace mineral and vitamin D therapies which are compatible and nutritionally available since this method would provide greater efficacy as compared to nutritional regimens known in the art. In addition, this nutritional regimen would be extremely useful as an additional or adjunct therapy for known drug treatments of bone disease and age-related bone loss, i.e. calcitonin, estrogen and didronel, including salts of amino functional compounds. Moreover, it would be quite useful to have such nutritional supplements which could be added to food and beverage compositions without undesirably affecting organoleptic and aesthetic properties.

It is an object of the present invention to provide calcium and trace mineral supplements which, when combined with vitamin D, and/or calcitonin, Didronel or estrogen, provide bone growth and can be used to treat age-related bone loss or to correct the imbalance that occurs between bone formation and bone resorption.

It is a further object of this invention to provide foodstuffs, beverages and beverage concentrates which are supplemented with calcium, trace minerals, vitamin D, and/or related drug therapies.

These and other objects are readily apparent from the description herein.

SUMMARY OF THE INVENTION

The supplements employ specific calcium salts of mixtures of citric and malic acids. The drug and/or vitamin therapies which they are combined with include vitamin D, Didronel (diphosphonates) and calcitonin. The mineral supplements comprise zinc, copper and manganese. Estrogen can be used in conjunction with any of these therapies.

The present invention provides methods for building bone in a human or other animal subject, comprising administering to said subject a safe and effective amount of vitamin D, didronel, estrogen and/or calcitonin, along with calcium citrate malate and copper, zinc and manganese salts. The calcium citrate malate comprises a complex or a mixture of calcium salts having a ratio of moles citrate to moles malate of from about 1:0.16 to about 1:13.5. The combination of these minerals and drugs is preferably administered in an oral dosage form, containing pharmaceutically-acceptable carriers and excipients.

All ratios, proportions and percentages herein are by weight, unless otherwise specified. All weights of the minerals are on an elemental basis unless otherwise specified.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to stable mineral/vitamin and/or drug supplements and supplemented foods and beverages including dry beverage mixes and to a method of building bone.

As used herein, the term "comprising" means various components can be conjointly employed in the mineral supplements, foods and beverages of the present invention. Accordingly, the terms "consisting essentially of" and "consisting of" are embodied in the term comprising.

By "nutritional" or "nutritionally-supplemental amount" herein is meant that the mineral and vitamin sources used in the practice of this invention provide a nourishing amount of the trace minerals, calcium and vitamin D. This is supplemental or in addition to the amount found in the diet. This supplemental amount will comprise at least 25% of the Recommended Dietary Allowance (RDA) of the daily intake of calcium, copper, manganese, zinc and vitamin D. Preferably, at least 50% of the Recommended Dietary Allowance (RDA) will be provided. The RDA for minerals and vitamins is as defined in The United States of America (see

Recommended Daily Dietary Allowance-Food and Nutrition Board, National Academy of Sciences-National Research Council).

Specific compounds and compositions to be used in these processes must, accordingly, be pharmaceutically acceptable. As used herein, a "pharmaceutically acceptable" component is one that is suitable for use with humans and/or animals without undue adverse side effects (such as toxicity, irritation, and allergic response) commensurate with a reasonable benefit/risk ratio. Further, as used herein, the term "safe and effective amount" refers to the quantity of a component which is sufficient to yield a desired therapeutic response without undue adverse side effects (such as toxicity, irritation, or allergic response) commensurate with a reasonable benefit/risk ratio when used in the manner of this invention. The specific "safe and effective amount" will, obviously, vary with such factors as the particular condition being treated, the physical condition of the patient, the duration of the treatment, the nature of concurrent therapy (if any), and the specific formulations employed.

As used herein, the term "flavors" includes both fruit and botanical flavors.

As used herein the term "sweeteners" includes sugars, for example, glucose, sucrose, and fructose. Sugars also include high fructose corn syrup solids, invert sugar, sugar alcohols, including sorbitol, and mixtures thereof. Artificial sweeteners are also included in the term sweetener.

As used herein, the term "trace minerals" means copper, manganese and zinc. These minerals play an important role in nutrition, but are required in only small or trace amounts in the diet. All three of these minerals are important enzymatic cofactors which are essential in development of bone in animals and humans. The trace minerals

herein are administered in the form of pharmaceutically acceptable salts.

The "carboxylate counterion" used in the preparation of the preferred mineral salts herein can be any ingestible carboxylate species. However, some judgement must be made with regard to flavor contribution. For example, citrate, malate and ascorbate yield ingestible complexes whose flavors are judged to be quite acceptable, particularly in fruit juice beverages. Tartaric acid is acceptable, particularly in grape juice beverages, as is lactic acid. Longer-chain fatty acids may be used in solid mineral supplements, but can affect flavor and water solubility. For essentially all purposes, the malate (preferred), gluconate, citrate and ascorbate moieties suffice, although others can be selected, according to the desires of the formulator.

The counterion for the trace minerals can also be phosphate, chloride, sulfate, nitrate or the like. However, these inorganic counterions can undesirably interact with calcium ions, especially in beverages. In high concentrations, these counterions, particularly chloride and sulfate, may contribute an undesirable flavor note to the supplement, food, or beverage containing them. Accordingly, the carboxylate counterions noted above are preferred herein.

Calcitonin

Calcitonin is a 32 amino acid protein produced by the thyroid C cells in higher mammals. It is known to block the stimulatory effects of PTH and other humoral agents on bone resorption. Calcitonin therapy decreases the rate of bone loss in osteoportoic patients. It is also used to treat Paget's disease of the skeleton and is used in combination with ethane dihydroxy diphosphonate. As used herein, the term calcitonin includes thyrocalcitonin, whether natural or synthetic.

Vitamin D

Vitamin D includes vitamin D, cholecalciferol (D_3), ergocalciferol (D_2) and its biolofically active metabolites and precursors such as, 1α, 25-$(OH)_2$ vitamin D; 25 OH vitamin D, its biologic precursor; and 1α hydroxy vitamin D, and analogues of the dihydroxy compound. These materials promote intestinal absorption of calcium, contribute to plasma calcium regulation by acting on bone density and stimulate reabsorption of calcium by the kidney.

Didronel

It is the disodium salt of 1-hydroxyethylidene diphosphonic acid (EHDP or editronate). It and other related diphosphonates and amino diphosphonates are collectively referred to herein as "diphosphonates".

Didronel and the diphosphonates act primarily on the bone. They inhibit the formation, growth and dissolution of hydroxyapatite crystals and their amorphous precursors by chemisorption to calcium phosphate surfaces. Generally, they do not adversely effect the levels of parathyroid hormones or calcium.

Bone-active drugs which are pharmaceutically-acceptable salts of amine-functional compounds include pharmaceutically-acceptable salts of amine-functional diphosphonate drug compounds and phosphonoalkylphosphinate drug compounds, including the prodrug esters thereof. Such compounds are disclosed, for example, in U.S. Patent Nos. 3,683,080 issued to Francis on August 8, 1972; 4,304,734 issued to Jary, Rihakova & Zobacova on December 8, 1981; 4,687,768 issued to Benedict & Johnson on August 18, 1987; 4,711,880 issued to Stahl & Schmitz on December 8, 1987; and 4,719,203 issued to Bosies & Gall on January 12, 1988; copending U.S. patent application Serial Nos. 808,584* of Benedict & Perkins filed December 13, 1985; 945,069* of Ebetino, Buckingham & McOsker filed December 19, 1986; 945,068* of Ebetino & Benedict filed December 19, 1986; and 069,666* of Ebetino filed July 6, 1987; and European Patent Application Nos. 0,001,584 of Blum, Hempel & Worms,

*(see page 32 for publication numbers)

published May 2, 1979; 0,039,033 published April 11, 1981; 0,186,405 of Benedict & Perkins, published July 2, 1986; and 0,243,173 of Oku, Todo, Kasahara, Nakamura, Kayakiri & Hashimoto, published October 28, 1987; all of which are hereby incorporated herein in their entirety by reference. Bone-active drugs more preferred in compositions of the present invention include pharmaceutically-acceptable salts of the following compounds or esters thereof: 6-amino-1-hydroxy-hexane-1,1-diphosphonic acid, 4-amino-1-hydroxy-butane-1,1-diphosphonic acid, N,N-dimethyl-4-amino-1-hydroxy-butane-1,1-diphosphonic acid, N,N-diethyl-4-amino-1-hydroxy-butane-1,1-diphosphonic acid, 3-amino-1-hydroxy-propane-1,1-diphosphonic acid, N,N-dimethyl-3-amino-1-hydroxy-propane-1,1-diphosphonic acid; N,N-diethyl-3-amino-1-hydroxy-propane-1,1-diphosphonic acid, 6-aminohexane-1,1-diphosphonic acid, phenylaminomethane diphosphonic acid, N,N-dimethylaminoethane diphosphonic acid, N-(2-hydroxyethyl)-aminomethane diphosphonic acid, N-acetylaminomethane diphosphonic acid, 3-(2-acetylaminocyclohexyl)-1-hydroxypropane-1,1-diphosphonic acid, 2 -(2-aminomethylcyclohexyl)-1-hydroxyethane-1,1-diphosphonic acid, cis-3-(2-aminocyclohexyl)-1-hydroxypropane-1,1-diphosphonic acid, 3-(1-aminocyclohexyl)-1-hydroxypropane-1,1-diphosphonic acid, 3 -(2-aminocyclohexyl)-1-hydroxypropane-1,1-diphosphonic acid, 3-(2-aminocyclopentyl)-1-hydroxypropane-1,1-diphosphonic acid, 1-aminoindan-2,2-diphosphonic acid, 4 -(aminomethyl)-indan-2,2-diphosphonic acid, 1-(aminomethyl)-dihydro-2-pyrindine-6,6-diphosphonic acid, 4-aminohexahydroindan-2,2-diphosphonic acid, 4 -(aminomethyl)-hexahydroindan-2,2-diphosphonic acid, octahydro-1-pyridine-5,5-diphosphonic acid; octahydro-2-pyridine-5,5-diphosphonic acid; octahydro-1-pyridine-6,6-diphosphonic acid; octahydro-2-pyridine-6,6-diphosphonic acid; octahydro-1-pyridine-7,7-diphosphonic acid; octahydro-2-pyridine-7,7-diphosphonic acid; 2-methyl-octahydro-1-

pyridine-5,5-diphosphonic acid; 1,3-diethyl-octahydro-2-pyridine-5,5-diphosphonic acid; 7-hydroxy-octahydro-1-pyridine-6,6-diphosphonic acid; 4-methoxy-octahydro-2-pyridine-6,6-diphosphonic acid; 5-vinyl-octahydro-1-pyridine-7,7-diphosphonic acid; 1 -(dimethylamino)-octahydro-2-pyridine-7,7-diphosphonic acid; N-acetyl-octahydro-2-pyridine-6,6-diphosphonic acid; N-benzyl-octahydro-1-pyridine-5,5-diphosphonic acid; N-(p-methoxyphenyl)-octahydro-2-pyridine-7,7-diphosphonic acid; 2-(3,4-dichlorophenyl)-octahydro-1-pyridine-7,7-diphosphonic acid; 2-(p-dimethylaminophenyl)-octahydro-1-pyridine-7,7-diphosphonic acid; 4-chloro-octahydro-1-pyridine-6,6-diphosphonic acid; 4-amino-octahydro-1-pyridine-6,6-diphosphonic acid; 7-carboxy-octahydro-1-pyridine-6,6-diphosphonic acid; 5-carboxy(methyl ester)-octahydro-1-pyridine-6,6-diphosphonic acid; 4-hydroxy-octahydro-2-pyridine-6,6-diphosphonic acid, propanoate ester; 4-(N,N-dimethylamino)-octahydro-1-pyridine-6,6-diphosphonic acid; 5 -(N-acetamido)-octahydro-1-pyridine-7,7-diphosphonic acid; 7-(ethylketone)-octahydro-2-pyridine-5,5-diphosphonic acid; and 4-nitro-octahydro-1-pyridine-6,6-diphosphonic acid; (2'-piperidinyl)-methane diphosphonic acid; (3'-piperidinyl)-methane diphosphonic acid; (4'-piperidinyl)-methane diphosphonic acid; 2-(2'-piperidinyl)-ethane-1,1-diphosphonic acid; 2-(3'-piperidinyl)-ethane-1,1-diphosphonic acid; 2 -(4'piperidinyl)ethane-1,1-diphosphonic acid; 2-(2'piperidinyl)-1-hydroxy-ethane-1,1-diphosphonic acid; 2-(3'-piperidinyl)-1-hydroxy-ethane-1,1-diphosphonic acid; 2-(4'-piperidinyl)-1-hydroxy-ethane-1,1-diphosphonic acid; 2-(2'-(3'methyl)-piperidinyl)-ethane-1,1-diphosphonic acid; 2-(2'-(5'-methyl)-piperidinyl)-ethane-1,1-diphosphonic acid; 2-(2'-(3'-methyl)-piperidinyl)-1-hydroxy-ethane-1,1-diphosphonic acid; 3 -(2'-piperidinyl)-propane-1,1-diphosphonic acid; 3 -(3'-piperidinyl)-propane-1,1-diphosphonic acid; 3 -(4'-piperidinyl)-propane-1,1-diphosphonic acid; 3

-(2′-piperidinyl)-1-hydroxy-propane-1,1-diphosphonic acid; 3-(3′-piperidinyl)-1-hydroxy-propane-1,1-diphosphonic acid; 3 -(4′-piperidinyl)-1-hydroxy-propane-1,1-diphosphonic acid; 3 -(2′-piperidinyl)-propane-2,2-diphosphonic acid; 3 -(3′-piperidinyl)-propane-2,2-diphosphonic acid; 3 -(4′-piperidinyl)-propane-2,2-diphosphonic acid; 2 -(2′-(N-methyl)-piperidinyl)-1-hydroxy-ethane-1,1-diphosphonic acid; 2-(2′-piperidinyl)-1-amino-ethane-1,1-diphosphonic acid; 2 -(3′-piperidinyl)-1-amino-ethane-1,1-diphosphonic acid; 2-(4′-piperidinyl)-1-amino-ethane-1,1-diphosphonic acid; 2-(2′-(3′-methyl)-piperidinyl)-1-amino-ethane-1,1-diphosphonic acid; 2-(2′-piperidinyl)-1-hydroxy-propane-1,1-diphosphonic acid; 3-(2′-piperidinyl)-propionic acid-2,2-diphosphonic acid; 2 -(2′-piperidinyl)-1-(N-methyl)amino-ethane-1,1-diphosphonic acid; 4-(2′-piperidinyl)-1-hydroxy-butane-1,1-diphosphonic acid; 2 -(2′-(5′-amino)-piperidinyl)-1-hydroxy-ethane-1,1-diphosphonic acid; 2-(2′-(3′-ethyl)-piperidinyl)-1-hydroxy-ethane-1,1-diphosphonic acid; 2-(2′-(3′-carboxy)-piperidinyl)-1-hydroxy-ethane-1,1-diphosphonic acid; (2-(2′-(5′-carboxy)-piperidinyl)-1-hydroxy-ethane-1,1-diphosphonic acid; 2-(2′-(1′,4′-diazinyl))-ethane-1,1-diphosphonic acid; 2-(2′-(1′,4′-diazinyl))-1-hydroxy-ethane-1,1-diphosphonic acid; 2-(2′-(1′,3′-diazinyl))-ethane-1,1-diphosphonic acid; 2-(3′-(1′,2′-diazinyl))-ethane-1,1-diphosphonic acid; N-(2′-piperidinylidene)-amino-methane diphosphonic acid; N-(3′-piperidinyl)-amino-methane diphosphonic acid; N-(4′-piperidinyl)-amino-methane diphosphonic acid; N-(2′-(3′-methyl)-piperidinylidene)-amino-methane diphosphonic acid; N-(2′-(5′-methyl)-piperidinylidene)-amino-methane diphosphonic acid; 2 -(N-(2′-piperidinylidene)-amino)-ethane-1,1-diphosphonic acid; 1-(N-(2′-piperidinylidene)-amino)-ethane-1,1-diphosphonic acid; N-(2′-(1′,3′-diazinylidene))-aminomethane diphosphonic acid;

N-(2'(1',4'-diazinylidene))-aminomethane diphosphonic acid; N-(2'-(1',3',5'-triazinylidene))-aminomethane diphosphonic acid; N-(4'-(1',2'-diazinyl))-aminomethane diphosphonic acid; O-(3'piperidinyl)-oxamethane diphosphonic acid; O-(4'-piperidinyl)-oxamethane diphosphonic acid; 2 -(O-(3'-piperidinyl)-oxa)-ethane-1,1-diphosphonic acid; 1 -(O-(3'-piperidinyl)-oxa)-ethane-1,1-diphosphonic acid; O-(4'-(1',2'-diazinyl))-oxamethane diphosphonic acid; S-(3'-piperidinyl)-thiomethane diphosphonic acid; S-(4'-piperidinyl)-thiomethane diphosphonic acid; 2 -(S-(3'-piperidinyl)-thio)-ethane-1,1-diphosphonic acid; 1-(S-(3'-piperidinyl)-thio)-ethane-1,1-diphosphonic acid; S-(4'-(1',2'-diazinyl))-thiomethane diphosphonic acid; N-(2-pyridyl)-aminomethane diphosphonic acid; N-(2-(5-amino)-pyridyl)-aminomethane diphosphonic acid; N-(2-(5-chloro)-pyridyl)-aminomethane diphosphonic acid; N-(2-(5-nitro)-pyridyl)-aminomethane diphosphonic acid; N-(2-(3,5-dichloro)-pyridyl)-aminomethane diphosphonic acid; N-(4-pyridyl)-N-ethyl-aminomethane diphosphonic acid; N-(2-(3-picolyl))-aminomethane diphosphonic acid; N-(2-(4-picolyl))-aminomethane diphosphonic acid; N-(2-(5-picolyl))-aminomethane diphosphonic acid; N-(2-(6-picolyl))-aminomethane diphosphonic acid; N-(2-(3,4-lutidine))-aminomethane diphosphonic acid; N-(2-(4,6-lutidine))-aminomethane diphosphonic acid; N-(2-pyrimidyl)-aminomethane diphosphonic acid; N-(4-(2,6-dimethyl)-pyrimidyl)-aminomethane diphosphonic acid; N-(2(4,6-dihydroxy)-pyrimidyl)-aminomethane diphosphonic acid; N-(2-(5-methoxy)-pyridyl)-aminomethane diphosphonic acid; N-(2-pyridyl)-2-aminoethane-1,1-diphosphonic acid; N-(2-(3-picolyl))-2-aminoethane-1,1-diphosphonic acid; N-(3-pyridyl)-2-amino-1-chloroethane-1,1-diphosphonic acid; N-(3-pyridyl)-2-amino-1-chloroethane-1,1-diphosphonic acid; N-(2-(4-picolyl))-2-amino-1-hydroxy-ethane-1,1-diphosphonic acid; (3-pyridyl)-aminomethane diphosphonic acid; 2 -(2-pyridyl)-1-amino-ethane-1,1-diphosphonic acid;

O-(4-pyridyl)-1-amino-2-oxa-ethane-1,1-diphosphonic acid; N-(phenyl)thiocarbamoylmethane diphosphonic acid; N-(4-chlorophenyl)carbamoylmethane diphosphonic acid; N-(4-chlorophenyl)thiocarbamoylmethane diphosphonic acid; N-(2-benzoyl[b]thienyl)thiocarbamoylmethane diphosphonic acid); N-(4-trifluoromethylphenyl)thiocarbamoylmethane diphosphonic acid); N-(3-trifluoromethylphenyl)thiocarbamoylmethane diphosphonic acid); N-(4-chloro-3-trifluoromethylphenyl)thiocarbamoylmethane diphosphonic acid); (4-chlorophenyl)sulfonylaminomethane diphosphonic acid); and N-(3-mesylaminophenyl)thiocarbamoylmethane diphosphonic acid). Bone-active drug compounds which are more preferred still in compositions of the present invention include pharmaceutically-acceptable salts of the following compounds or esters thereof: 6-amino-1-hydroxy-hexane-1,1-diphosphonic acid, 3-amino-1-hydroxy-propane-1,1-diphosphonic acid, octahydro-1-pyridine-6,6-diphosphonic acid, 2-(2'-piperidinyl)-ethane-1,1-diphosphonic acid; 2 -(3'-piperidinyl)-ethane-1,1-diphosphonic acid; 2 -(2'-piperidinyl)-1-hydroxy-ethane-1,1-diphosphonic acid; 2-(3'-piperidinyl)-1-hydroxy-ethane-1,1-diphosphonic acid; N-(2'-(3'-methyl)-piperidinylidene)-amino-methane diphosphonic acid; N-(2'-(1',3'-diazinylidene))-aminomethane diphosphonic acid; and N-(2-(3-methylpiperidinylidene))-aminomethane-1,1-diphosphonic acid. Other preferred bone active drug compounds for use in the compositions of the present invention include the phosphonomethyl phosphinic acid compounds which are analogous to the above diphosphonic acid compounds.

As used herein, a "pharmaceutically-acceptable salt of an amine-functional compound" means a salt of addition of the compound having similar pharmacological activity (efficacy and safety) to the base form of the compound. Preferably a salt of addition of a compound has substantially the same pharmacological activity as the base form of the

compound. A salt of addition of a compound can be formed by the association of an amine-functional drug compound in its base form with an appropriate acid. Acids known to form pharmaceutically-acceptable salts of addition with base forms of certain amine-functional drug compounds include, but are not limited to, the following: hydrochloric, hydrobromic, hydriodic, maleic, succinic, tartaric, fumaric, lactic, citric, ascorbic, oxalic, gluconic, phosphoric, nitric, sulfuric, methane sulfonic, ethane sulfonic and 2-naphthalene sulfonic.

<u>Estrogen</u>

Estrogen therapy can be used along with any of these regimens. The method herein also comprises coadministering from about 0.3 mg to about 6 mg of estrogen along with the calcium and trace minerals and vitamin D, calcitonin or editronate (or diphosphonates). Preferably from 0.625 mg to about 1.25 mg of estrogen is taken daily. Any viable estrogen hormone replacement can be used.

<u>Calcium Trace Mineral Component</u>

In supplements of the type disclosed herein, the nutritionally supplemental amount for the minerals will generally comprise more than 50% of the RDA and preferably 80%-100% RDA, most preferably 100% of the RDA, per unit portion of the finished supplement. Of course, it is recognized that the preferred daily intake of any mineral may vary with the user.

In general, the RDA (calcium) will range from 360 mg per 6 Kg for infants to 800 mg/54-58 Kg female, depending somewhat on age. Moreover, it can be difficult to supplement beverages with more than 20-30% RDA of calcium (based per serving) without encountering precipitation and/or organoleptic problems. However, this level of supplementation is equivalent to cow's milk in calcium value, and is therefore acceptable.

The recommended dietary allowance for zinc is 15 milligrams (mg) per day for males and 12 mg per day for

females. There is no specific RDA for manganese and copper. A safe and adequate range has been established as 2 to 5 mg for manganese and for copper, the range is 1.5 mg to 3 mg per day.

Any soluble salt of the trace minerals can be used, for example, zinc chloride, zinc sulfate, manganese sulfate, manganese gluconate, copper sulfate and copper gluconate are useful. A nutritionally supplemental amount of these minerals is used. However, the particular salt used and the level will depend upon their interaction with other supplement ingredients.

Inorganic anions which are useful for making the trace mineral salts are sulfate, nitrate, phosphate, hydrogen phosphate and carbonate. Organic anions include carboxylate anions, e.g. citrate, malate tartrate, acetate and glyconate.

It is essential to this supplementation that the calcium salts be soluble in the stomach. This solubilization aids in making the calcium more readily bioavailable. It is equally important that the trace minerals be solubilized and absorbed by the stomach and or intestine. Therefore the choice of calcium and mineral salts depends upon the interaction of the salts in acid (stomach pH) solutions or basic (intestinal pH) solutions.

Solubility also plays an important role in the preparation of foods and beverages containing these supplements.

<u>Calcium Citrate Malate Compositions</u>:

The methods of this invention involve administration of a mixture of calcium salts, herein "calcium citrate malate," comprising calcium salts of citric acid and malic acid. The calcium citrate malate may consist of a mixture of calcium citrate and calcium malate, a complex of calcium containing citrate and malate ligands, a mixture of a calcium salt with citric acid and malic acid, or combinations thereof. Mixtures of a calcium salts and citric and malic acids may be used to form calcium citrate malate <u>in</u>

situ, the beverage. Preferred are calcium citrate malate mixtures made by adding calcium carbonate, calcium hydroxide or other suitable source to a mixture of citric and malic acids.

The molar ratio of citrate:malate is from about 1:0.16 to about 1:13.5, preferably from about 1:0.5 to about 1:4.5, more preferably from about 1:0.75 to about 1:3. The ratio of moles calcium:total moles citrate:total moles of malate is from about 2:1:1 to about 8:2:1, preferably from about 4:2:3 to about 6:3:4. The calcium citrate malate may contain other acid anions in addition to citrate and malate. Such anions may include, for example, carbonate, hydroxide, phosphate and mixtures thereof depending on the calcium source.

Preferably, the calcium citrate malate is neutral, comprised entirely of citrate and malate anions. Thus, preferably, the equivalents of calcium (2 x moles calcium) is about equal to the total number of equivalents of citrate (3 x moles citrate) plus malate (2 x moles malate). A preferred calcium citrate malate has a calcium:citrate:malate molar composition of about 6:2:3 and 4:2:3.

The calcium citrate malate used in the methods of this invention may be provided in solid or liquid forms. Calcium citrate malate for use in solid forms may be made, for example, by first dissolving citric acid and malic acid, in the desired molar ratio, in water. Calcium carbonate may then be added to the solution, in such amount that the ratio of moles calcium to moles citrate and moles malate is as desired. Carbon dioxide will be evolved. The solution may then be dried (as by freeze drying or oven drying at temperatures below 100°C) to obtain the calcium citrate malate. Methods for making calcium citrate malate are described in the following documents: Japanese Patent Specification SHO 56-97248, Kawai, published August 5, 1981; and in U. S. 4,722,847 issued to Heckert (1988). Co-pending application of Fox et al, Serial Number 07/537313*

(* see page 32 for publication number)

filed June 14, 1990; Japanese Patent Specification SHO 56 -97248, Kawai, published August 5, 1981; and in U.S. 4,722,847 issued to Heckert (1988).

Calcium carbonate can be used as the calcium source. Other sources include calcium oxide and calcium hydroxide. Calcium chloride, calcium phosphate and calcium sulphate are suitable for use herein, but they can form an acid solution which could adversely affect the flavor of beverages and water solutions of the calcium citrate malate.

A solid forms during the mixing of the calcium oxide or calcium hydroxide with the citric and malic acid. When these materials are used, it is necessary to mix the solution until all of the calcium appears to have dissolved. The calcium citrate malate ligand will precipitate when its solubility is exceeded.

The preferred method of preparation is to prepare a highly concentrated solution of the calcium citrate malate which quickly and efficiently forces metastable calcium citrate malate out of solution. Concentrations of from 20% to 75% are preferred. Preferably the concentration is from 40% to 65%.

The reaction temperature can be ambient (20°C) or higher. Preferably the temperature of the reaction is in the range of 30°C to 80°C. Most preferably it is from 40°C to 60°C.

Flavor Component

The flavor component of the present invention contains flavors selected from natural flavors, bot ical flavors and mixtures thereof. The term "fruit flavors" refers to those flavors derived from the edible reproductive part of a seed plant, especially one having a sweet pulp associated with the seed. Also included within the term "fruit flavor" are synthetically prepared flavors made to simulate fruit flavors derived from natural sources.

The term "botanical flavor" refers to flavors derived from parts of a plant other than the fruit; i.e. derived

from bean, nuts, bark, roots and leaves. Also included within the term "botanical flavor" are synthetically prepared flavors made to simulate botanical flavors derived from natural sources. Examples of such flavors include cocoa, chocolate, vanilla, coffee, kola, tea, and the like. Botanical flavors can be derived from natural sources such as essential oils and extracts, or can be synthetically prepared.

The particular amount of the flavor component effective for imparting flavor characteristics to the supplements and food or beverage mixes of the present invention ("flavor enhancing") can depend upon the flavor(s) selected, the flavor impression desired, and the form of the flavor component. The flavor component can comprise at least 0.05% by weight of the beverage composition and preferably from 0.05% to about 10%. The amount of flavor added to the food, beverage or supplement is within the skill of one in the art and depends on the flavor intensity desired.

For chocolate or cocoa, the amount of flavor added is from about 0.05% to about 20%. Lower levels of artificial or synthetic chocolate flavors are used than for cocoa itself.

Beverages can be flavored with fruit or other botanical flavors, e.g., vanilla, strawberry, cherry, pineapple, banana, and mixtures thereof.

The calcium, citric and malic acids can be added with the trace minerals and vitamin D to a 100% fruit juice or a diluted fruit juice. The sugars present in the juice are useful sweeteners, and the juice can be the flavor component. Such beverages can contain from 5% to 100% juice. Preferably dilute juice beverages will have from 10% to 40% juice. Preferred juices for 100% juice products or diluted products are orange, cranberry, apple, pear, grape, raspberry, lemon, grapefruit, pineapple, banana, blackberry, blueberry and passion fruit juices and mixtures thereof.

Sweetener Component

The sweetener composition is usually a monosaccharide or a disaccharide. These include sucrose, fructose, dextrose, maltose and lactose. Other carbohydrates can be used if less sweetness is desired. Mixtures of these sugars can be used.

In addition to sugar of the present invention can contain other natural or artificial sweeteners. Other suitable sweeteners include saccharin, cyclamates, acetosulfam, L-aspartyl-L-phenylalanine lower alkyl ester sweeteners (e.g. aspartame), L-aspartyl-D-alanine amides disclosed in U.S. Patent 4,411,925 to Brennan et al., issued October 23, 1983, L-aspartyl-D-serine amides disclosed in U.S. Patent 4,399,163 at Brennan et al., issued August 16, 1983, L-aspartyl-L-1-hydroxymethyl- alkaneamide sweeteners disclosed in U.S. Patent 4,338,346 to Brand, issued December 21, 1982, L-aspartyl-1-hydroxyethyl-alkaneamide sweeteners disclosed in U.S. Patent 4,423,029 to Rizzi, issued December 27, 1983, L-aspartyl-D-phenylglycine ester and amide sweeteners disclosed in European Patent Application 168,112 to J. M. Janusz, published January 15, 1986, and the like. A particularly preferred sweetener is aspartame.

The amount of the sweetener effective in the food, beverage, mixes or supplements of the invention depends upon the particular sweetener used and the sweetness intensity desired. For noncaloric sweeteners, this amount varies depending upon the sweetness intensity of the particular sweetener. For sugar (i.e., sucrose), this amount can be from 10% to 85% (typically from 55% to 70%) by weight. In determining the amount of sugar, any sugar or other sweetener present in the flavor component is also included. Low-calorie sweetener combinations containing a noncaloric sweetener such as aspartame and a sugar, such as corn syrup solids, or sugar alcohols can also be used in

beverage mixes. In general, the amount of sweetener will be from about 0.5% to about 85%.

Other Ingredients

Other minor ingredients are frequently included in supplements, foods and beverages. Such ingredients include preservatives such as benzoic acid and salts thereof, sulfur dioxide, butylated hydroxyanisole, butylated hydroxytoluene, etc. Also, typically included are colors derived either from natural sources or synthetically prepared.

Salt, e.g. sodium chloride, and other flavor enhancers can be used to improve the flavor of the food, beverage or supplement.

Emulsifiers can also be included. Any food grade emulsifier can be used. Lecithin is a preferred emulsifier. Other edible emulsifiers include mono- and diglycerides of long chain fatty acids, preferably saturated fatty acids, and most preferably, stearic and palmitic acid mono- and diglycerides. Propylene glycol esters are also useful in beverage mixes.

Fats or oils can also be added to supplements or foods to make them more palatable.

pH and Other Beverage Ingredients

The pH of the beverages and beverage concentrates of the present invention is dependent upon the weight ratios of the acids, the total amount of acids and the sourness impression desired. Typically, the pH can range from 2.5 to 6.5. Preferred carbonated beverages have a pH of from 3.0 to 4.5.

Other minor beverage ingredients are frequently included in beverages and concentrates. Also, typically included are colors derived either from natural sources or synthetically prepared. See L. F. Green, Developments in Soft Drinks Technology, Vol. 1 (Applied Science Publishers Ltd. 1978), pp. 185-186 (herein incorporated by reference) for preservatives and colors used in beverages.

Beverage Preparation

The beverages and concentrates of the present invention can be prepared by standard beverage formulation techniques. It should be understood, however, that carbonated beverage making techniques, when appropriately modified, are also applicable to noncarbonated beverages. Also, while the following description is with reference to sugar containing beverages, diet beverages containing noncaloric sweeteners can also be prepared by appropriate modification. Beverages can include dry beverage mixes which are made by mixing flavors, sweeteners, and other optional ingredients as well as fruit juices and dilute fruit juices.

In making a sugar sweetened carbonated beverage, a beverage concentrate is usually formed containing from 30 to 70% by weight water. This beverage concentrate typically contains the emulsified or water-soluble flavors, emulsion stabilizing agents, and weighting agents if needed, any color desired and suitable preservatives. After the concentrate is formed, sugar and water are then added to make a beverage syrup. This beverage syrup is then mixed with an appropriate quantity of water to form the finished beverage. The weight ratio of water:syrup is from about 3:1 (3x syrup) to about 5:1 (5x syrup). To make a carbonated beverage carbon dioxide can be introduced either into the water mixed with the beverage syrup or into the drinkable diluted beverage to achieve carbonation. The beverage can be sealed in a container such as a bottle or can. See L.F. Green, Developments in Soft Drinks Technology, Vol. 1, (Applied Science Publishers Ltd. 1978), pp. 102-107 (herein incorporated by reference), for a further description of beverage making, in particular the process for carbonation.

The amount of carbon dioxide in the beverage depends upon the particular flavor system used and the amount of carbonation desired. Usually, carbonated beverages of the present invention contain from 1.0 to 4.5 volumes of carbon

dioxide. Preferred carbonated beverages contain from 2 to 3.5 volumes of carbon dioxide.

The calcium source and the acids (citric, malic, phosphoric) can be added at various points in these processes. The calcium source and acids are preferably added at the same point in this process, but can also be added at different points. Usually, the calcium source and acids are included during preparation of the beverage concentrate or beverage syrup. Preferably the trace minerals are added after the calcium and acid source have been mixed in. Vitamin D can be added with the oil flavors or weighting oil.

When making a dry beverage, it is preferred to mix a powdered calcium citrate malate powder and trace mineral salts with the sugar or artificial sweeteners, vitamin D and flavors. Colors and colored coated sugars can be added. Dry chocolate milk beverages are preferred dry beverage mixes. These can be diluted either with water or milk. Milk provides additional vitamin D and calcium citrate. A typical formula for chocolate mixes is:

a) from 0% to 25% milk solids, preferably from 5% to 20% non-fat milk solids;

b) from 0.05% to 20% flavor, preferably cocoa;

c) from about 0.5 to about 85% sweetener, preferably sucrose; and

d) from about 0.6% to about 0.15% calcium citrate malate and from about 0.60 to about 30 micrograms vitamin D.

<u>Supplement Forms</u>

Solid forms include tablets, capsules, granules and bulk powders. Tablets may contain suitable binders, lubricants, diluents, disintegrating agents, coloring agents, flavoring agents, flow-inducing agents and melting agents. Liquid oral dosage forms include aqueous solutions, emulsions, suspensions, solutions and/or suspensions reconstituted from non-effervescent granules and effervescent

preparations reconstituted from effervescent granules. Such liquid oral dosage forms may contain, for example, suitable solvents, preservatives, emulsifying agents, suspending agents, diluents, sweeteners, melting agents, and coloring and flavoring agents. A preferred liquid dosage form contains calcium citrate malate and the trace minerals in a juice-containing beverage or other beverage.

The trace minerals, calcium citrate malate, vitamin D and/or and drug therapy can be coadministered in one tablet, liquid, food or beverage or they can be administered separately. A capsule containing the trace mineral salts, a second tablet with the calcium citrate malate and a third containing vitamin D and/or drug therapy are easy to formulate and to swallow. A mineral and vitamin D supplement could also be coadministered with a calcium beverage.

Specific examples of pharmaceutically acceptable carriers and excipients that may be used to formulate oral dosage forms of the present invention are described in U. S. Patent 3,903,297, Robert, issued September 2, 1975 Techniques and compositions for making dosage forms useful in the methods of this invention are described in the following references; 7 Modern Pharmaceutics, Chapters 9 and 10 (Banker & Rhodes, Editors, 1979); Lieberman et al., Pharmaceutical Dosage Forms: Tablets (1981); and Ansel, Introduction to Pharmaceutical Dosage Forms 2nd Edition. (1976).

Method of Building Bone

Various oral dosage forms of calcium citrate malate, trace minerals, vitamin D and/or drug therapies may be used in the present invention. Such dosage forms comprise a safe and effective amount of calcium citrate malate, trace minerals, vitamin D and/or drug therapies and a pharmaceutically acceptable carrier. Preferably the pharmaceutically acceptable carrier is present at a level of from about 0.1% to about 99%, preferably from about 0.1% to about 75%, by weight of the composition. Unit dosage forms (i.e.,

dosage forms containing an amount of calcium citrate malate suitable for administration in one single dose, according to sound medical practice) preferably contain from about 100 mg to about 1000 mg, preferably from about 100 mg to about 500 mg, more preferably from about 200 mg to about 300 mg of calcium (on an elemental basis). Unit dosage forms of copper (on an elemental basis) contain 0.5 to 5 mg, preferably 0.5 to 4.0 mg, of manganese (on an elemental basis) contain 1 to 8 mg and preferably from 2 mg to 7 mg, and of zinc (on an elemental basis) contain 1.5 to 30 mg and preferably 7.5 to 20 mg zinc.

Preferably, from about 175 milligrams to about 2000 milligrams of calcium (as elemental calcium) are administered to said subject, per day. More preferably, from about 250 milligrams to about 1500 milligrams, most preferably from about 350 milligrams to about 1000 milligrams, of calcium are administered, per day. The specific amount of calcium citrate malate to be administered depends upon the relative percentage weight of calcium in the particular calcium citrate malate employed.

The recommended daily allowance for vitamin D is 200-400 IU depending on age of the subject. The supplements used herein have a unit dosage amount of from about 25 IU to about 1000 IU or from about 0.6 to 25 micrograms.

The effective amount of calcitonin is about 100 IU per day. From about 25 IU to 120 IU can be administered with the calcium citrate malate, trace minerals and vitamin D.

The effective amount of diphosponate is from about 200 mg to about 1000 mg. The usual safe and effective amount of Didronel and the diphosphonates is from 1 mg/kg/day to 20 mg/kg/day. Preferably from 5 to 10 mg/kg/day are used. This dose can be administered in 2 dosage units, one in the morning and 1 in the evening. Didronel and the bisphosphonates for treatment of age-related bone loss are typically administered in an intermittent cyclical fashion.

Specifically, the present invention provides a method for building bone in a human or other animal subject, comprising administering to said subject a safe and effective amount of calcium citrate malate, and copper, zinc and manganese and vitamin D and/or either calcitonin, editronate or estrogen for a period of time sufficient to achieve an increase in the net skeletal mass of said subject. As used herein, "building bone" refers to a decrease in the net skeletal loss of bone of the subject treated and therefore a net skeletal increase in mass. The slowing of the rate of bone loss and the increase in growth rate occur simultaneously so the net bone density may stay the same. The increase in mass may be at any skeletal site, including spine, hip, long bones of arms or legs or in the whole skeleton. Preferably, the net skeletal mass is increased by at least about 0.1%, more preferably at least about 1%.

The loss of bone is cumulative over a long period of time. Typically, lifetime loss in bone mass is about 35% in males and 50% in females. Thus, even though a net skeletal increase of as little as 0.5% in one year is not particularly critical, over 10 years this results in 5% more bone mass than would be present if bone loss continued at its usual rate.

"Administering" refers to any method which, in sound medical practice, delivers the vitamin D and/or drug, calcium citrate malate and trace minerals used in this invention to the subject to be treated in such a manner so as to be effective in the building of bone.

The specific period of time sufficient to achieve an increase in the net skeletal mass of the subject may depend on a variety of factors. Such factors include, for example, the specific mineral formulation employed, the amount of minerals administered, the age and sex of the subject, the specific disorder to be treated, concomitant therapies employed (if any), the general physical health of the subject (including the presence of other disorders), the

extent of bone loss in the individual, and the nutritional habits of the individual. Although the administration of even small quantities of calcium citrate malate, trace minerals, vitamin D and/or drug therapy may build bone, the net increase in bone mass may not be detectable for short periods of administration.

For the treatment of age-related bone loss, the calcium citrate malate, trace minerals and vitamin D, and/or calcitonin or bisphosphonates or estrogen are administered according to sound medical practice for at least about six months, preferably for at least about twelve months. Of course, such administration may be continued indefinitely, according to sound medical practice.

The methods of this invention may be employed in the treatment of any of a variety of disorders in which the building of bone is desired. Thus, preferably, the human or other animal "subject" of the methods of this invention is "in need" of a method for building bone, i.e., the subject has a disorder for which building of bone or decrease in rate of bone resorption would be advantageous according to sound medical practice. Such disorders include, for example, bone fractures, reduced bone mass and disorders typified by bone loss, such as age-related bone loss and osteoporosis (both primary and secondary forms).

A preferred method of this invention is for the treatment of age-related bone loss.

The following example illustrates compositions of the type provided by the practice of this invention, but is not intended to be limiting thereof.

Example I

Several post-menopausal women are treated by administering a composition containing calcium citrate malate having a molar calcium:citrate:malate composition of about 6:2:3. The calcium citrate malate is made by first dissolving approximately 384.2 grams of citric acid and approximately 402.3 grams of malic acid in approximately 2

liters of water. This citrate/malate solution is then heated to approximately 55°C (131°F), with stirring. Separately, approximately 600.6 grams of calcium carbonate is added to approximately 1.2 liters of water, forming a slurry, with stirring.

The citrate/malate solution is then removed from its heat source, and the calcium carbonate slurry is added slowly, with stirring. The rate of addition is controlled, to contain the reaction as carbon dioxide is released. An additional quantity of water, approximately 0.4 liters, is finally added. The reaction mixture is then stirred for approximately 1 to 1.5 hours. The reaction is essentially complete as the pH of the solution equilibrates to approximately 4.3.

A precipitate of calcium citrate malate is thus formed. The excess reaction liquid is filtered off. The calcium citrate malate is dried, for approximately 12 hours at approximately 105°C (221°F), reducing the moisture level to less than about 1%. The dried product is then milled to approximately 10-20 mesh size, for a swallowable tablet formulation. Each tablet contains 250 mg.

The swallowable tablet dosage form is then made, comprising:

Component	% (By Weight)
Calcium citrate malate*	99.73
Magnesium stearate	0.27

*Having a molar calcium:citrate:malate composition of approximately 6:2:3, made as described above in this example.

The tablet formulation is made by thoroughly admixing the powders, and tabletting using a standard tablet press, to form tablets weighing approximately 1104 milligrams. The tablets are then coated, using a pan coater. The coating solution contains approximately 11% hydroxypropylmethyl cellulose, approximately 2%

polyethylene glycol, approximately 3.5% colorant, and the balance of water.

A capsule containing 15 mg zinc (from zinc sulfate), 5 mg manganese (from manganese gluconate) and 2.5 mg copper (from copper gluconate) and 10 micrograms of vitamin D is also administered to each patient.

Example II

A powdered mineral supplement comprising 2000 gm of calcium citrate malate, 6.3 mg of copper sulfate (2.5 mg copper), 31.3 mg of zinc chloride (15 mg zinc) and 5 mg of manganese (15.4 mg of manganese sulfate monohydrate) is prepared by tabletting the mixture of powders. This tablet is taken in a daily regimin with 2 mg/kg of Didronel for 6 months.

Example III

A tablet containing 200 mg calcium (from calcium citrate malate), 1 mg copper (from copper gluconate), 1.5 mg manganese (from manganese gluconate), 3 mg zinc (from zinc gluconate), and 3 micrograms vitamin D (from cholecalciferol) is taken 4 times daily along with a single tabletted dose of 0.625 mg conjugated estrogen taking once daily for 2 years.

(* Publication Numbers:)

07/562,773	published 20 February 1992 as W092-02235
808,584	published 29 July 1987 as EP 0,230,068
945,069	published 06 July 1988 as EP 0,273,514
945,068	published 13 July 1988 as EP 0,274,158
069,666	published 11 January 1989 as EP 0,298,553
07/573,313	published 26 December 1991 as W091-19692

CLAIMS:

1. A mineral and vitamin supplement for building bones comprising a unit dosage mixture of:

a) from 100 to 1000 mg of calcium (on an elemental basis) in the form of a carboxylate selected from the group consisting of citrate, malate, lactate and mixtures thereof, preferably, wherein the molar ratio of calcium:citrate:malate is from 2:1:1 to 8:2:1;

b) from 0.5 to 5 mg of copper (on an elemental basis);

c) from 1.5 to 30 mg of zinc (on an elemental basis);

d) from 1 to 8 mg of manganese (on an elemental basis); and

e) from 0.6 to 25 micrograms of vitamin D, preferably wherein vitamin D is present at 0.15 to 5 micrograms per supplement and is in the form of vitamin D metabolites and precursors such as $1\alpha25\text{-}(OH)_2$ vitamin D, 25 OH vitamin, $1\alpha OH$ vitamin D_2 or D_3 and/or analogues of the dihydroxy compound.

2. A supplement according to Claim 1 wherein said copper, zinc and manganese are salts of anions selected from the group consisting of chloride, sulfate, gluconate, citrate, malate, lactate, tartrate, nitrate, and mixtures thereof.

3. A supplement for building bones comprising a unit dosage mixture of:

a) from 100 to 1000 mg of calcium (on an elemental basis) in the form of a carboxylate selected from the group consisting of citrate, malate, lactate and mixtures thereof;

b) from 0.5 to 5 mg of copper (on an elemental basis);

c) from 1.5 to 30 mg of zinc (on an elemental

basis);

d) from 1 to 8 mg of manganese (on an elemental basis); and

e) from 200 mg to 1000 mg of diphosphonate, preferably wherein said diphosphonate is Didronel or an aminodiphosphonate.

4. A method for building of bone in a human subject suffering from age-related bone loss comprising administering to said subject a mineral supplement comprising calcium citrate malate, zinc, manganese and copper salts and a member or members selected from the group consisting of calcitonin, vitamin D, editronate and diphosphonates for a sufficient period of time to build bone in said subject, wherein said calcium citrate malate is administered at a level of from 175 milligrams to 2000 milligrams (on an elemental calcium basis), per day, said zinc is administered at a level of 1.5 to 30 mg/day, said manganese at a level of 1 to 8 mg/day and said copper is at a level of 0.5 to 5.0 mg/day; said vitamin D is at a level of 0.6 to 25 micrograms/day and said diphosphonate is at a level of 200 to 1000 mg/day.

5. A method for building of bone according to Claim 4, wherein said period of time is at least six months, preferably wherein said period of time is sufficient to increase the net skeletal mass of said subject by at least 0.5%.

6. A method for building of bone according to Claim 4 or 5, wherein said calcium citrate malate has a molar ratio of citrate:malate of from 1:0.5 to 1:4.5 and wherein said zinc is from 7.5 to 20 mg and said copper is from 0.5 to 5 mg and said manganese is from 2 to 7 mg, preferably wherein said zinc is in the form of zinc sulfate and said manganese and copper are in the form of manganese gluconate and copper gluconate.

7. A method for building of bone according to Claim 4, 5 or 6 wherein said mineral supplement is in a solid dosage form.

8. A method for building of bone according to Claim 4, 5 or 6 wherein said mineral supplement is in a liquid dosage form, preferably, a beverage and preferably wherein said beverage contains juice selected from the group consisting of orange juice, apple juice, pear juice, grape juice, lemon juice and cranberry juice.

9. A method according to Claim 7 wherein said solid dosage form comprises a tablet of calcium citrate malate and vitamin D and a separate tablet or capsule of zinc, copper and manganese salts.

10. A method for building bone according to Claim 1 wherein a safe and effective amount of estrogen is also administered to the human subject, preferably wherein said estrogen is administered in unit doses of from 0.6 mg to 6 mg.

INTERNATIONAL SEARCH REPORT

International application No. PCT/US 92/03995

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5 A 61 K 33/06 A 61 K 33/30 A 61 K 33/32
A 61 K 33/34 A 61 K 31/59 A 61 K 31/565 A 23 L 1/304
A 61 L 1/303

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	A 61 K A 23 L

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category [°]	Citation of Document,[11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
Y	EP,A,0343703 (PROCTER & GAMBLE) 29 November 1989, see claims 1-7; examples III,VI ---	1-10
Y,P	WO,A,9202235 (PROCTER & GAMBLE) 20 February 1992, see page 15, line 34 - page 16, line 17; page 16, line 30 - page 17, line 11; page 19, lines 15-24; claims (cited in the application) ---	1-10
Y	J.E.F. REYNOLDS et al.: "Martindale the Extra Pharmacopoeia", 28th edition, 1982, pages 1657-1659, The Pharmaceutical Press, London, GB, see the whole document ---	1-10
A	EP,A,0300895 (SYNTHELABO) 25 January 1989, see claims --- -/-	1-9

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
03-09-1992	21.09.92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	Mme Dagmar FRANK

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	GB,A,2196523 (BEECHAM GROUP) 5 May 1988, see page 1, lines 41-42; claims ---	1-9
A	Patent Abstracts of Japan, vol. 13, no. 532 (C659), 28 November 1989, & JP,A,1218547 (SNOW BRAND MILK PROD.) 31 August 1988, see abstract ---	1-9
A	US,A,3992556 (L.E. KOVACS et al.) 16 November 1976, see column 4; table (cited in the application) -----	1-9

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9203995
SA 60577

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 16/09/92
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0343703	29-11-89	AU-A- 3516089	30-11-89
		JP-A- 2072843	13-03-90
WO-A- 9202235	20-02-92	AU-A- 8503591	02-03-92
EP-A- 0300895	25-01-89	FR-A- 2618305	27-01-89
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		DE-A- 3864621	10-10-91
		JP-A- 1067164	13-03-89
GB-A- 2196523	05-05-88	None	
US-A- 3992556	16-11-76	US-A- 3992555	16-11-76

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82